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Journal:	*Plant Biosystems*
Manuscript ID	TPLB-2017-0110.R2
Manuscript Type:	Original Article
Date Submitted by the Author:	20-Jul-2017
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Keywords:	Pimpinella anisum L., essential oil, fatty acids, phenolic, antioxidant activity, provenance

Comparative assessment of phytochemical profiles and antioxidant properties of Tunisian and Egyptian anise (*Pimpinella anisum* L.) seeds

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Abstract

Anis (*Pimpinella anisum* L.) seeds obtained from two geographic origins Tunisia (TAS) and Egypte (EAS) were studied regarding their biochemical composition and the antioxidant potential of their extracts. The results showed that the highest value of oil was detected with TAS compared to that of EAS ones. Ten (10) fatty acids were identified for the two locations and petroselinic acid was the most prevalent in oil seeds and interestingly, TAS displayed a significantly higher level of this acid than EAS. Besides, TAS exhibited slightly higher essential oil yield than the Egyptian variety and that *trans*-anethole was the dominant for the two provenances. In both accessions, the highest total phenolic, flavonoid and tannin content was obtained with ethyl acetate fraction. Therefore, TAS exhibited higher chelating and reducing powers than EAS which may be due to a slightly different phenolic composition between the two accession seed extracts. The phenolic compositions of TAS and EAS revealed that ethyl acetate extracts showed higher proportions of naringin, chlorogenic acid and rosmarinic acid. However, ethanol extracts were richer in larcitrin, rosmarinic acid and cirsimartin. The overall results revealed that aniseeds might constitute a novel source of natural antioxidants and could be used as food additive.

Keywords

Pimpinella anisum L.; essential oil; fatty acids; phenolic; antioxidant activity; provenance.

Introduction

The World Health Organization estimates that about 80% of the developing countries inhabitants rely on the traditional medicine for their primary health care needs, and that most of these therapies involve the use of plant extracts or their active components (WHO, 2000). Not only in developing countries but all over the world the use of medicinal plants has been playing a significant role in maintaining human health and improving the quality of human life. Thus, Fruits have become important for human nutrition due to their nutrients and potential beneficial health effects (Albuquerque et al. 2016).

Pimpinella anisum L. (*P. anisum*) has been widely used as a culinary ingredient as well as traditional remedies for the treatment of different disorders in the folk medicine systems of different civilizations. Aniseed contains 1.5–6.0 mass % of a volatile oil consisting primarily of *trans*-anethole and also as much as 8–11 mass % of lipids rich in fatty acids, such as palmitic and oleic acids, as well as approximately 4 mass % of carbohydrates, and 18 mass % of protein (Besharati-seidani et al. 2005). Anise essential oil is mainly constituted by anethole, an aromatic substance that appears as the major compound of the oil, usually corresponding to more than 80% (w/w) of the oil (Samojlik et al. 2012; Özel 2009; Ullah and Honermeier 2013). Thus, seeds of anise are commonly recommended as antioxidant, antiseptic, antimicrobial, aperitif, digestive, antispasmodic (in respiratory and gastrointestinal tracts), expectorant, galactogogue, estrogenic, anti-inflammatory and diuretic agents, being these benefits mainly associated with the essential oil (Boskabady and Ramazani-Assari 2001; Shojaii and Fard 2012). Moreover, the oleochemical industry is increasingly interested in custom-made and novel oils with specific fatty acid compositions for applications in the oil and pharmaceutical industries (Murphy 1999). Such oils can be used for the synthesis of high-quality products without expensive purification of raw materials. For the assessment of the

nutritional and economical value of oilseeds the knowledge on the compositional factors is very essential in connection with the properties (Ramadan and Wahdan 2012). Moreover, application of synthetic antioxidants in food processing has led to the appearance of remarkable side effects (Ebrahimabadi et al., 2010). Due to these limitations, there is an increasing interest in finding naturally and biologically produced antioxidants capable of inhibiting free radical reactions, retarding oxidative rancidity of lipids, protecting the human body from diseases, and preserving foods from spoiling (Terao and Piskula 1997). What's more, the antioxidant potential of plants was generally determined by the phenolic compounds, being promoters of wellbeing and life expectancy of individuals (Li et al., 2014). A few reports describe the phenolic profile of aniseeds (Marques and Farah 2009; Martins et al. 2016). Thus, the composition of phenolic fractions present in *P. anisum* seeds is still incompletely studied and some data are contradictory. Hence, in this study, we evaluated for the first time the biochemical properties and the antioxidant potential of Tunisian aniseed fractions and try to compare them with the Egyptian ones. Further, characterization of active principle is needed to understand the effect of geographic origin on the chemical composition of *P. anisum* seeds and so to improve their economic and health utilization as a source of natural bioactive compounds.

Materials and methods

Plant material and growth conditions

Two accessions of mature aniseeds (*Pimpinella anisum* L.) were used in this work. The first called (TAS) were harvested in June 2015 from the region of Korba in the northeast of Tunisia; latitude 36340 38.22''(N); longitude 10510 29.63''(E) and the altitude is 637 m. The precipitation average was 400-500 mm/year and the monthly average temperature was 17.7 C. The other seeds were reported to be imported from Egypt (EAS).

Plant identification was carried by Professor Abderrzek Smaoui (Biotechnology Center in Borj-Cedria Technopole, Tunisia). A voucher specimen was deposited at the herbarium of the Laboratory of Bioactive Substances, Biotechnology Center in Borj-Cedria Technopole under the "BC2011-2002" number. The two provenances were cultivated under the same environmental conditions. Thus, seeds were transplanted to 10 l pots filled with agricultural soil which had a clayey–loamy texture and were irrigated with tap water. Experiments were carried out in a greenhouse with a 14 h photoperiod (photosynthetic photon flux density, PPFD: 400 mol m^{-2} s^{-1}) and lasts 3 months from February 2016 to April 2016. Mean temperature and relative humidity were, respectively, 30 ± 5 °C, 55 ± 5% day and 16 ± 2 °C, 90 ± 5% night. After harvest, seed were air-dried and stored at 4 °C until use for further analysis.

Oil extraction

Aniseeds were finely ground in an electric grinder (IKA-WERK. Type: A: 10). 10 g of each ground sample were extracted using a soxhlet-apparatus with 100 mL hexane (Analytical Reagent, LabScan, Ltd., Dublin, Ireland) for 6 h. The extraction was protected against light. Oil was removed after mixture filtration and solvent evaporation under reduced pressure.

Total lipid extraction

Total lipids of aniseeds were extracted by the modified method of Bligh and Dyer (1959), according to Marzouk and Cherif (1981).

Fatty acid methylation and analysis

Total fatty acids were converted into their methyl esters using 3% sodium methylate in methanol according to the method described by Cecchi et al. (1985).

Essential oil extraction

Aniseed (ripe and dried fruit of *Pimpinella anisum* L.) were finely ground in an electric grinder (IKA-WERK. Type: A: 10). Triplicate samples of 100 g were subjected to hydrodistillation in 1 L of deionized water using a Clevenger apparatus for up to 4 h, time which was necessary for a complete extraction.

Gas Chromatography (GC) analysis

GC analysis of volatile compounds was carried out according to Zaouali et al. (2010) using an Agilent 6980 gas chromatograph equipped with a flame ionisation detector (FID) and an electronic pressure control (EPC) injector attached to HP-INNOWAX polyethylene glycol capillary column (30 m 0.25 mm). The flow of the carrier gas (N^2) was 1.6 mL min^{-1}. The split ratio was 60:1. The analysis was performed using the following temperature program: oven temps isotherm at 35 °C for 10 min, from 35 to 205 °C at the rate of 3 °C min^{-1} and isotherm at 205 °C during 10 min. Injector and detector temperature were held, respectively, at 250 and 300 °C. One micro-liter of the sample (dissolved in hexane as 1/50 v/v) was injected into the system. Individual peaks were identified by comparison of their retention indices relative to (C6-C22) n-alkanes with those of literature and/or with those authentic compounds available in our laboratory. Percentage compositions of samples were calculated according to the area of the chromatographic peaks using the total ion current.

Gas Chromatography-Mass Spectrometry (GC-MS)

The identification of the EOs was performed using a Hewlett Packard HP5890 series II GC–MS equipped with a HP5MS column (30 m 0.25 mm). The carrier gas was helium at 1.2 mL min1. Each sample (1µL) was injected in the split mode (1:20), the program used was isothermal at 70°C, followed by 50–240°C at a rate of 5°C min1, then held at 240°C for 10 min. The mass spectrometer was an HP 5972 and the total electronic impact mode at 70 eV was used. The components were identified by comparing their relative retention times and

mass spectra with the data from the library of EOs constituents, Wiley, Mass-Finder and Adams GC–MS libraries.

Polyphenol extraction and analysis

Preparation of extracts

The plant extract was prepared as described earlier by Zahin et al. (2010). Briefly, two hundred (200) grams of dry aniseed powder was soaked in 1 L of hexane 24 h with intermittent shaking and at the end of extraction the extract was filtered through Whatman filter paper no.1 (Whatman Ltd., England) to make an hexane fraction (HF). The same dried powder of seeds was further taken for fractionation with the same above procedure with dichloromethane to obtain dichloromethane fraction (DF). After extraction, the same material was successively extracted with ethyl acetate ethanol to obtain EAF and EF, respectively. The filtered fractions were concentrated to dryness under reduced pressure on rotary evaporato at 40°C and stored at 4°C for future use.

Total phenolic amounts

The total phenolic amount of the extracts was determined by using Folin-Ciocalteu reagent (Merck), according to the procedure described by Dewanto et al. (2002).

Total flavonoids content

Total flavonoid contents (TFC) were measured according to Dewanto et al. (2002).

Assessment of Total Condensed Tannins

Total tannin contents were measured using the modified vanillin assay described by Sun et al. (2002).

DPPH radical scavenging assay

Radical-scavenging activity was determined according to Hanato et al. (1988).

Chelating effect on ferrous ions

The ferrous ion chelating activity of aniseed extracts was assessed as described by Zhao et al. (2006).

Reducing power

The method of Oyaizu (1986) was used to assess the reducing power of different seed extracts.

RP-HPLC evaluation of phenolic compounds

Diluted samples from *P. anisum* seeds were injected to RP-HPLC. The separation of phenolics was performed with an Agilent 1100 series HPLC system equipped with on-line degasser (G 1322A), quaternary pump (G 1311A), a thermostatic auto sampler (G 1313A), column heater (G 1316A) and diode array detector (G 1315A). Instrument control and data analysis were carried out using Agilent HPLC Chemstation 10.1 edition through Windows 2000. The separation was carried out on a reverse phase ODS C18 (4 µm, 2509 4.6 mm, Hypersil) column used as stationary phase at ambient temperature. The mobile phase consisted of acetonitrile (solvent A) and water sulphuric acid (0.2%) (solvent B). The flow rate was kept at 0.5 mL min-1. The gradient program was as follows: 15 A/85% B 0–12 min, 40% A/60% B 12–14 min, 60% A/40% B 14–18 min, 80% A/20% B 18–20 min, 90% A/10% B 20–24 min, 100% A 24–28 min. The injection volume was 20 µl and peaks were monitored at 280 nm. Peak identification was obtained comparing the retention time and the UV spectra of the *P.anisum* phenolics chromatogram with those of pure standards which were purchased from Sigma (St. Louis, MO, USA). Analyses were performed in triplicates

Statistical analysis

Data were subjected to statistical analysis using statistical program package STATISTICA. Percentage of each parameter was the mean of six replicates ± S.D and the differences between individual means were deemed to be significant at $p<0.05$.

Results and discussion

Oil yield and fatty acid composition

Nowadays, research has increased to investigate new plant sources of oil from underexploited seeds. Thus, the oils obtained in this experiment, were extracted from *P. anisum* seeds with *n*-hexane in soxhlet apparatus. The highest value was detected with Tunisian aniseeds (TAS) with 11.60% compared to that of Egyptian ones (EAS) with 9.82% (Table 1). These values give *P. anisum* nutritional and industrial importance. We did not find information about oil accumulation in TAS and EAS, but our results were similar to other authors who reported that Brazilian and Polandian aniseeds contained 5-11 mass % of lipids rich in fatty acids (Besharati-Seidani et al. 2005; Kozlowska et al. 2016) and that the oil content could be fluctuated with geographic origin. Generally, it has been known that *Apiaceae* crops contained a noticeable yield of oil ranged from 8% to 24% (Reiter et al. 1998). As summarized in Table 1, Ten (10) fatty acids were identified. Results showed that the monounsaturated fatty acid (MUFA) proportion was the predominant (67.65% and 56.87% respectively, for TAS and EAS). Among MUFA, petroselinic acid was the most prevalent in oil seeds and interestingly, the Tunisian variety displayed a significantly higher level of this acid (46.60%) than the Egyptian one (38.40%). This is in agreement with the Kleiman and Spencer (1982) and Denev et al. (2011) findings in American and Bulgarian aniseeds. Furthermore, aniseed oil obtained from the two provenances also contained oleic (C18:1 Δ9) acid with the proportion exceeding 18%. Aniseed oil was also characterized by an important level of linoleic acid (C18:2). These two fatty acids play an important role in cell

components and were used by the personal care products industry due to its beneficial properties for skin (Tlili et al. 2014). Moreover, the amount of saturated fatty acids (SFA) in these oils was considerably low, 6.57 for TAS and 14.50 for EAS and represented mainly by palmitic acid (C16:0). Typical of the *Apiaceae* plant is that the major fatty acid component in the seed oils is petroselinic acid, instead of oleic acid. However, Kozlowska et al. (2016) demonstrated that the fatty acid profile for Polandian aniseeds, in which petroselinic acid was absent, was different from the fatty acid profile of the aniseed analyzed in our study. Also, Matthäus et al. (2014) reported that linoleic acid (59.3%) was determined as the major constituent of Turkish aniseeds which is totally different from our findings. Previous reports have suggested that genetic factor as well as environment were a source of variability of fatty acids (Bettaieb et al. 2010). Generally, as indicator of nutritional importance, the fatty acid composition also determines the value of edible oils. Indeed, petroselinic acid is of potential industrial significance. It can be oxidatively cleaved to produce a mixture of lauric acid, a compound useful in the production of detergents, and adipic acid, a C6 dicarboxylic acid that can be used in the synthesis of nylon polymer (Murphy 1999).

Essential oil content and composition

In the present study, the analysis of essential oil content of anise (Suppemental Figure S1) showed that TAS exhibited slightly higher yield than the Egyptian variety (2.43 and 1.72% respectively). These values were in agreement with previously published results (Tabanca et al. 2005; Tepe et al. 2006; Ullaha and Honermeiera 2013). Therefore, it could be concluded that Tunisian aniseeds meet the demand of the European Pharmacopeia (European Pharmacopoeia, 2000).

On the other hand, the chemical composition of the aniseed essential oil was markedly similar according the two provenances (Table 2). Fourteen compounds were determined and representing 99% and 97% of the total oil respectively for TAS and EAS.

The compounds of analyzed essential oil are grouped in 4 chemical classes according to their functional groupings. Indeed, phenylpropanoides are represented in high amount (95%, approximately), followed by sesquiterpene hydrocarbons. On the other hand, oxygenated and terpenic hydrocarbons were the minor class in aniseed essential oil.

In current studies, *trans*-anethole was the dominant constituent which proportion varied from 94.30 to 90.41%, respectively for TAS and EAS. This component has a sweet herbaceous odour, sweet taste and was largely used as a substrate for synthesis of various pharmaceutical substances (Kosalec et al. 2005).

Other compounds were characterized the essential oil profiles of aniseeds such as γ-himachalene (2.32-1.08%), estragole (0.20-3.74%), β-bisabolene (0.19-0.85%), diepi-α-cedrene (0.91-0.08%), respectively for TAS and EAS (Table 2). Indeed, even the same main compounds were present in the two varieties; there was a great difference in their percentages and this can be due to environmental and genetic factors (Bettaieb Rebey et al. 2016).

Based on the previous reports carried out on aniseed oils, *trans*-anethole, γ-himachalene and estragole are the characteristic compounds for *Pimpinella anisum* essential oils (Tabanca et al. 2005; Tepe et al. 2006; Ullaha and Honermeiera 2013). Thus, Singh et al. (2008) mentioned that nine chemical constituents were found by gas chromatography and mass spectrometry (GC-MS) analysis from the essential oil of Indian aniseed and that the major constituent was *trans*-Anethole (90.1%) and Fenchone (5%). Besides, the higher amount of *trans*-Anethole (96.80%) was reported in essential oil of Serbian aniseeds by Acimovic et al. (2015). Furthermore, Fitsiou et al. (2016) determined that the main components of the anise essential oil were *trans*-Anethole (88.1%) followed by γ-himachalene (4.15%), and cis-isogenol (4.15%). While, Al- Maofari et al. (2013) demonstrated that 4-allylanisole was the major compound of *Pimpinella anisum* L. essential oil. Fortunately, *cis*-anethole, which is toxic, was not detected in our essential oil, while it was detected in anise essential oil from other origins

(Ullah and Honermeier 2013; Acimovic et al. 2015; Fitsiou et al. 2016). On the other hand, the yield of aniseed may noticeably vary depending on ecological conditions such as temperature, precipitation and soil fertility (Ullah and Honermeier 2013) (Supplemental Table S1).

Total phenolic, flavonoid and tannin contents

It was evident that aniseeds contained noticeable amounts of phenolic content ranged from 31.22 to 1.82 for TAS and 17.43 to 1.03 for EAS (Supplemental Figure S2). Total phenolic contents extracted from TAS were significantly higher compared to EAS. In both accessions, the highest total phenolic content was obtained with ethyl acetate, followed by ethanol, dichloromethane and hexane fractions. According to Shobha et al. (2013), the total phenolic content of ethyl acetate extract from Indian aniseeds was higher than other solvent extracts. This result is in agreement with the report of Scholz and Rimpler (1989) who showed ethyl acetate is often used as an extraction solvent with a significant selectivity in the extraction of low-molecular-weight phenolic compounds and high-molecular-weight polyphenols methanol as the most suitable solvent for extraction of phenolic compounds. Contrary to these results, Gülçin et al. (2003) reported that the ethanol extract of Turkish aniseeds had highest amount of total phenolic compounds (77.5 mg GAE/g DW) compared to the water extract (30 mg GAE/g DW). Bagdassarian et al. (2013) reported that total phenolic content evaluated in Bulgarian aniseed methanolic extract was 46.17 mg/g DW. These changes could be ascribed to the variations in pedoclimatic conditions. Additionally, the ethyl acetate extract of aniseeds obtained from Tunisian provenance showed higher polyphenol content than the Egyptian one, suggesting that phenolic biosynthesis in *P. anisum* is greatly influenced by genetic factors as mentioned by Bettaieb et al. (2012) in the case of *Cuminum cyminum* seeds.

Total flavonoid content of aniseeds varied from 2.76 to 48.52 mg CE/g DW for TAS and from 1.88 to 31.08 mg CE/g DW for EAS. There were significant differences in total flavonoid

concentration among the two accessions. Total flavonoid contents extracted from TAS were higher than those from EAS. Regarding flavonoid solubility, the solvent classification with respect to their extraction efficiency was similar to that made for polyphenols having an order of ethyl acetate>ethanol>dichloromethane>hexane. Shobha et al. (2013) also showed that ethyl acetate is an efficient solvent for extracting flavonoids from aniseeds.

As found for phenolics and flavonoids, condensed tannin contents were found to vary depending on the solvent used. Condensed tannin contents were less abundant than flavonoid contents in aniseeds obtained by different solvents The highest condensed tannin contents were recorded when extraction was achieved using ethyl acetate (5.11 mg EC/g DW) for TAS and ethanol (4.29 mg EC/g DW) for EAS (Supplemental Figure S3). Shobha et al. (2013) reported that n-butanol was more efficient than ethyl acetate to extract condensed tannins for Indian aniseeds.

As matter of fact, it is also important to note that genetic and geographic factors, culture conditions, climatic changes, harvesting time, storage and manipulation procedures, among others, should significantly affect the composition of phenolic and, consequently, the biological potential and their use as healthy promoters.

Antioxidant activity

Various studies have focused on natural antioxidants in plant extracts and their applications in food systems to prevent oxidation. The most widely used synthetic antioxidants in food (butylated hydroxytoluene BHT, butylated hydroxyanisole BHA) are very effective in their role as antioxidants. However, their use in food products has been failing off due to their instability or their suspected action as promoters of carcinogenesis (Namiki 1990). For this reason, there is a growing interest in the studies of natural healthy (nontoxic) additives as potential antioxidants (Tomaino et al. 2005).

P. anisum extracts exhibited variable abilities to quench DPPH radical as a function of the solvent type (Supplemental Figure S4). Ethanol and dichloromethane extracts of EAS showed the highest abilities to scavenge DPPH radical with IC_{50} = 12.58 and 16.45 µg/mL, respectively. This activity was more potent than that of the well known synthetic antioxidant BHT (IC50 = 24.12 µg/ml). In addition, Ethyl acetate extract of TAS had higher potential to scavenge DPPH radical (IC_{50} = 18.75 µg/mL) than the positive control BHT. The lowest antiradical capacity was found in hexane extracts of aniseeds with IC_{50} = 168.25 µg/mL for TAS and 194.32 µg/mL for EAS. Nickavar and Al Sadat Abolhasani (2009) reported that the radical scavenging activities of Iranian aniseeds were mainly intense for ethyl acetate extract, followed by water, chloroform and hexane extract. Gülçin et al. (2003) mentioned that the ethanol and water extracts of Turkish aniseeds had lower antiradical potential than the positive controls (BHT, BHA and α-tocopherol). These significant differences in antioxidant potential between solvent systems were essentially due to the difference in polarity, and thus different extractability of the antioxidative compounds (Ksouri et al. 2008).

The effect of solvent on the antioxidant abilities of TAS and EAS was also assessed by the estimation of chelating and reducing powers estimation (Table 3). TAS exhibited higher chelating and reducing powers than EAS which may be due to a slightly different phenolic composition between the two accession seed extracts. The different extracts of both aniseed accessions showed power antioxidant activities, but ethyl acetate led to the highest chelating power (IC_{50} = 9.73 mg/mL for TAS and 33.65 mg/mL for EAS) and the lowest reducing capacity (EC_{50} = 510.22 mg/mL for TAS and 687 mg/mL for EAS). It was also observed that despite the inability of the *P. anisum* seed extracts to compete with the positive controls (ascorbic acid in iron reducing and EDTA in iron chelating), these extracts did possess mild antioxidant activities and may be considered as potential preservatives for food utilization where aniseeds were preferred due to its safety. Gülçin et al. (2003) also reported that the

ethanol and water extracts of Turkish aniseeds had lower chelating and reducing powers than the positive controls (BHT, BHA and α-tocopherol).

Moreover, from the results of present study, it is evident that the antioxidant activities of *P.anisum*, are related to various phenolic compounds present in one or more fractions. In general, the higher polyphenols extraction yield corresponds with the higher antioxidant activity, probably due to the combined action of the substances present in variable concentrations and their high hydrogen atom donating abilities. Similarly, a linear correlation between DPPH radical scavenging activity and polyphenolic extract has been reported as variable ranges in different food plants (Siddhuraju and Becker 2003)

Identification of phenolic compounds using HPLC

Generally, phenolic compounds act as important contributors to the antioxidant potential of plant extracts. So, their characterization could provide considerable benefits to individuals, mainly through inciting their use as healthy promoters.

In this context, ethyl acetate and ethanol were the most efficient solvents to extract phenolics for TAS and EAS accessions. Despite these two accessions contained identical phenolic compounds, qualitative and quantitative differences were found between the two solvent extracts (Table 4). For the two accessions, Ethyl acetate and ethanol extracts contained more flavonoids (55.57% and 72.70% for TAS and 55.81% and 73.29% for EAS, respectively) than phenolic acids (45.97% and 25.26% for TAS and 41.56% and 24.43% for EAS, respectively). A total of 15 phenolic compounds were identified. The phenolic compositions of TAS and EAS revealed that ethyl acetate extracts showed higher proportions of naringin (32.12% for TAS and 33.33% for EAS), chlorogenic acid (29.37% for TAS and 24.18% for EAS) and rosmarinic acid (10.90% for TAS and 10.32 for EAS). However, ethanol extracts were richer in larcitrin (25.26% for TAS and 26.87% for EAS), rosmarinic acid (18.54% for TAS and 20.59% for EAS) and cirsimartin (13.97% for TAS and 17.62% for EAS).

Variations in phenolic composition between the two solvent extracts could be explained by the difference in polarity, and thus different extractability, of the antioxidative compounds (Djeridane et al. 2006; Maisuthisakul et al. 2007). Several studies showed that solvent polarity leads to significantly different extraction capacities for phenolic compounds in plants (Parida et al. 2004; Galvez et al. 2005). Quantitative analysis of total phenolic compounds using HPLC indicated that ethyl acetate extract contained more total phenolics (10.18 mg/g for TAS and 7.68 mg/g for EAS) than ethanol extract (7.44 mg/g for TAS and 5.73 mg/g for EAS). However, phenolic contents obtained by HPLC were significantly lower than those obtained by the spectrophotometrical method. This was predictable due to the low selectivity of Folin-Ciocalteu reagent, as it reacts positively with different phenolic and non-phenolic substances (Que et al. 2006). Martins et al. (2016) quantified the total phenolic comnpounds of *P. anisum* seeds by HPLC having 42.09 mg/g and they qualified phenolic composition counting six hydroxycinnamic acid derivatives and ten flavones derivatives mainly luteolin and apigenin derivatives. In earlier study of Kunzemann and Herrmann (1977), isolation and structure elucidation of flavonoid constituents from anise spice by means of chromatography on cellulose columns lead to isolation of quercetin 3-glucuronide, rutin, luteolin 7-glucoside, isoorientin, isovitexin apigenin 7-glucoside and luteolin glycoside. Shobha et al. (2013) reported the abundance of apigenin and luteolin in ethyl acetate fraction of aniseeds. However, Zielinski et al. (2014) reported the richness of anise tea extract in chlorogenic acid and quercetin as found in ethyl acetate aniseed extract of our work.

The results presented here constitute the first information on the phytochemical composition and antioxidant activities of aniseed fractions of Tunisian and Egyptian accessions. Aniseed antioxidant activity was high enough for the plant to be a new and natural source of antioxidant substances for its use as natural additives in food. To understand their mechanism of action as bioactive components, further fractionation of ethyl acetate and ethanol extracts,

isolation of phenolic compounds and determination of their biological activities *in vitro* and *in vivo* are needed.

Acknowledgements

The authors thank Pr. Abderazzak Smaoui for identification of the plant.

Supplemental data

Supplemental data (Table S1, Figure S1, Figure S2, Figure S3, Figure S4, and Figure S5) can be accessed at supplementary materials section.

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Table 1 Oil yield and fatty acid composition (%) of Tunisian and Egyptian anise (*Pimpinella anisum*) seeds (Means of six replicates ± S.D). Values with different superscripts (a–b) are significantly different at $p < 0.05$.

	TAS	EAS
Oil yield (%)	11.60±0.03[a]	9.82±0.02[b]
Saturated fatty acid (SFA) (%)		
Capric acid (C10:0)	0,16±0.03[a]	0,11±0.01[a]
Lauric acid (C12:0)	0,52±0.01[a]	0,44±0.02[a]
Myristic acid (C14:0)	0,07±0.01[a]	0,02±0.00[a]
Palmitic acid (C16:0)	4,90±0.22[b]	13,20±0.09[a]
Stearic acid (C18:0)	0,85±0.04[a]	0,66±0.01[a]
Arachidic acid (C20:0)	0,07±0.01[a]	0,07±0.00[a]
Total	6.57	14.5
Unsaturated fatty acid (UFA) (%)		
Petroselinic acid (C18:1 Δ6)	46,60±0.22[a]	38,40±0.11[b]
Oleic acid (C18:1 Δ9)	21,05±0.08[a]	18,47±0.13[b]
Linoleic acid (C18:2)	22,99±0.44[a]	23,18±0.22[a]
Linolénic acid (C18:3)	1,07±0.01[a]	0,58±0.04[b]
Total	91.71	80.63

Values with different superscripts (a–b) are significantly different at $p < 0.05$ (means of six replicates); SFA: saturated fatty acid; UFA: unsaturated fatty acid.

Table 2 Essential oil composition of Tunisian and Egyptian anise (*Pimpinella anisum*) seeds (Means of six replicates ± S.D). Values with different superscripts (a–b) are significantly different at $p < 0.05$.

Compounds*	RI[a]	RI[b]	%	
			TAS	EAS
Terpene hydrocarbons			0.13	0.04
Linalool	1097	1557	0.13± 0.01[a]	0.04± 0.04[b]
Oxygenated Monoterpene			0.06	0.02
α-Terpinene	1018	1249	0.06±0.01[a]	0.02±0.00[a]
Phenylpropanoids			95.78	94.99
Anisole	918	1720	0.97±0.05[a]	0.52±0.03[b]
Estragole	1197	1430	0.20 ± 0.03[b]	3.74 ± 0.13[a]
trans-Anethole	1253	1740	94.30±0.01[a]	90.41±0.22[b]
p-Anisaldehyde	1250	1718	0.17 ± 0.01[a]	0.10 ± 0.07[a]
Cis-Isoeugenol	1359	2180	0.14±0.01[a]	0.22±0.02[a]
Sesquiterpene hydrocarbons			3.95	2.48
β-Elemene	1388	1465	0.07 ± 0.66[a]	0.09 ±2.11[a]
γ-Himachalene	1484	1690	2.32 ± 0.04[a]	1.08±0.01[b]
Zingiberene	1494	1672	0.30± 0.03[a]	0.25 ±0.03[a]
β-Himachalene	1505	1942	0.12±0.02[a]	0.11±0.01[a]
β-Bisabolene	1506	1832	0.19±0.02[b]	0.85±0.01[a]
Isolongifolene	1532	2003	0.04±0.00[a]	0.02±0.00[a]
Diepi-α-cedrene	1575	2020	0.91±0.02[a]	0.08±0.00[b]
Total identified			99.74	97.53

Volatile compounds percentages in the same line with different letters (a–b) are significantly different at P < 0.05(means of six replicates). RI[a] Order of elution in apolar column (HP-5); RI[b] Order of elution in polar column (HP Innowax), MS: mass spectrum.

Table 3. Antioxidant activities of TAS and EAS extracts

	Chelating ability (IC_{50}. mg/mL)		Reducing power (EC_{50}. µg/mL)	
	TAS	**EAS**	**TAS**	**EAS**
Ethanol	19.05 ± 0.38^{Ab}	55.46 ± 0.25^{Bb}	273.45 ± 0.55^{Ba}	454.63 ± 0.54^{Aa}
Ethyl acetate	9.73 ± 0.87^{Aa}	33.65 ± 0.83^{Ba}	510.22 ± 1.94^{Ac}	$687\pm1.77A^{Bc}$
EDTA	0.03±0.01		-	
Ascorbic acid	-		42±0.84	

Each value in the table was obtained by calculating the average of three experiments; The data marked with the different capital letter for the provenance and small letter for the solvents. in the table of each IC_{50} or EC_{50} value share significant differences at $P< 0.05$ (Duncan test).

Table 4. Phenolic compounds of ethyl acetate and ethanol extracts from Tunisian and Egyptian aniseeds

	Ethyl acetate				Ethanol			
	TAS		EAS		TAS		EAS	
	mg/mL	%	mg/mL	%	mg/mL	%	mg/mL	%
Phenolic acid	**4.68^{a}**	**45.97^{a}**	**3.18^{b}**	**41.56^{b}**	**1.88^{c}**	**25.26^{c}**	**1.40^{d}**	**24.43^{c}**
Gallic acid	0.01±0.00^{a}	0.09±0.01^{B}	0.02±0.01^{a}	0.26±0.01^{B}	0.01±0.01^{a}	0.13±0.02^{B}	0.07±0.01^{a}	1.22±0.02^{A}
Chlorogenic acid	2.99±0.01^{a}	29.37±0.22^{A}	1.85±0.01^{b}	24.18±0.02^{B}	-	-		
Caffeic acid	-	-	-	-	0.20±0.02	2.68±0.03	0.05±0.01^{a}	1.22±0.02^{a}
Syringic acid	0.03±0.01^{b}	0.29±0.08^{B}	0.10±0.01^{a}	1.30±0.01^{A}	-	-		
p-Coumaric acid	0.53±0.00^{a}	5.20±0.01^{A}	0.28±0.01^{b}	3.66±0.07^{B}	0.21±0.03^{b}	2.82±0.05^{C}	0.04±0.01^{c}	0.69±0.01^{D}
Rosmarinic acid	1.11±0.01^{b}	10.90±0.01^{C}	0.79±0.01^{c}	10.32±0.05^{C}	1.38±0.04^{a}	18.54±0.02^{B}	1.18±0.03^{a}	20.59±0.07^{A}
Ellargic acid	0.02±0.01^{c}	0.19±0.01^{b}	0.14±0.01^{a}	1.83±0.01^{a}	0.08±0.01^{ab}	1.07±0.04^{b}	0.06±0.01^{b}	1.04±0.01^{a}
Flavonoids	**5.25^{a}**	**51.57^{C}**	**4.27^{b}**	**55.81^{C}**	**5.41^{a}**	**72.70^{A}**	**4.20^{b}**	**73.29^{B}**
Epicatechin-3-Ɵ-gallate	0.14±0.01^{c}	1.37±0.02^{C}	0.07±0.01^{c}	0.91±0.01^{CD}	0.64±0.03^{a}	8.60±0.04^{A}	0.39±0.03^{b}	6.80±0.09^{B}
Coumarin	0.64±0.01^{a}	6.28±0.01^{A}	0.56±0.01^{b}	7.32±0.03^{A}	-	-		
Rutin	0.12±0.02^{c}	1.17±0.05^{D}	0.19±0.01^{c}	2.48±0.04^{C}	0.82±0.01^{a}	11.02±0.06^{A}	0.55±0.01^{b}	9.59±0.05^{B}
Quercetin	0.51±0.03^{c}	5.00±0.02^{B}	0.41±0.01^{c}	5.35±0.06^{B}	0.99±0.02^{a}	13.30±0.01^{A}	0.69±0.02^{b}	12.04±0.02^{A}
Naringin	3.27±0.03^{a}	32.12±0.01^{A}	2.55±0.01^{ab}	33.33±0.01^{A}	0.04±0.00^{b}	0.53±0.01^{B}	0.02±0.00^{b}	0.34±0.02^{B}
Apigenin	0.57±0.00^{a}	5.59±0.01^{AB}	0.49±0.01^{a}	6.40±0.02^{A}	-	-	-	
Larcitrin	-	-			1.88±0.07^{a}	25.26±0.08^{A}	1.54±0.03^{a}	26.87±0.05^{A}
Cirsimartin	-	-			1.04±0.05^{a}	13.97±0.04^{B}	1.01±0.02^{a}	17.62±0.04^{A}
NI	0.25±0.01^{a}	2.45±0.03^{b}	0.27±0.01^{a}	3.52±0.03^{a}	0.15±0.02^{a}	2.01±0.02^{A}	0.13±0.01^{a}	2.26±0.02^{B}
Total	**10.18**	**100**	**7.65**	**100**	**7.44**	**100**	**5.73**	**100**

Values are means of six replications (N=±6 SD). The data marked with capital letters (A-D) and small letters (a-d) in the same line indicate significant differences at $P < 0.05$ (Duncan test).

Comparative assessment of phytochemical profiles and antioxidant properties of Tunisian and Egyptian anise (*Pimpinella anisum* L.) seeds

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Supplementary materials

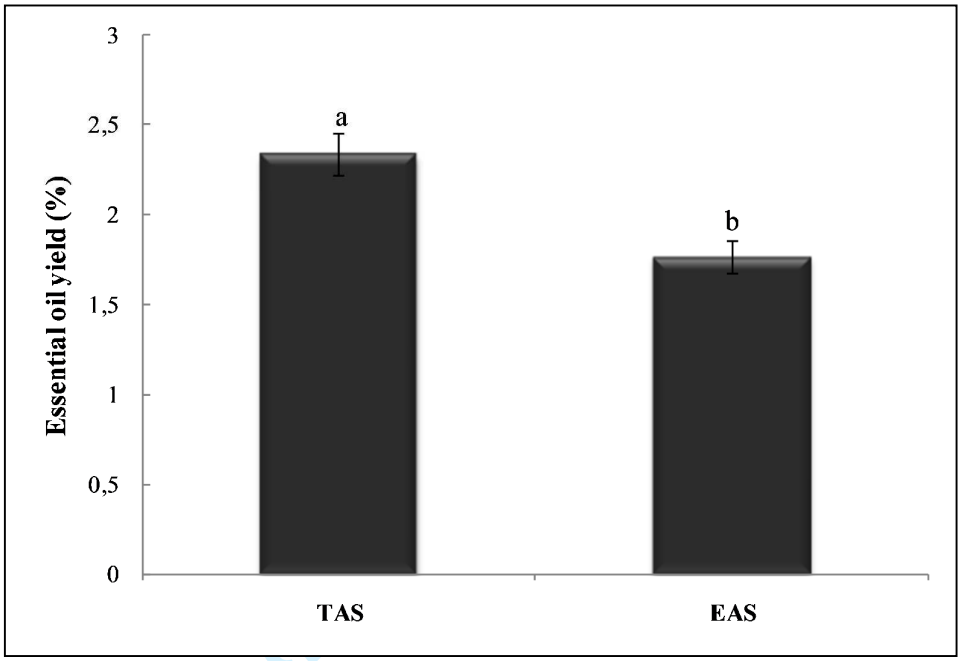


Figure S1. Essential oil yields of Tunisian and Egyptian anise (*Pimpinella anisum*) seeds (Means of six replicates ± S.D). Values with different superscripts (a–b) are significantly different at $p < 0.05$.

Table S1. Comparative table between the main volatile compounds (%) detected in *Pimpinella anisum* seeds cultivated in different countries.

	India	Serbia		Marocco		Yemen	Pakistan	Egypte	Greek	Turkey	Sudan
Volatile compounds (%)	Singh et al. (2008)	Samojlik et al. (2012)	Acimovic et al. (2015)	Ghouati et al. (2012)	Al Moafri et al (2013)	Al Moafri et al (2013)	Ullah and Honermeir, (2013)	AbdRaheem & Oraby (2015)	Fitsiou et al. (2016)	Tepe et al. (2016)	Hassan and Elhassan (2017)
***trans*-Anethole**	**90.1**	**88.49**	**96.8**	**81.19**	7.40	3.54	**84.07**	**82.1**	**88.1**	**82.8**	**78.21**
cis-Anethole	-	-	-	-	-	-	0.18	5.8	0.43	-	-
α-Himachalene	-	3.13	1.84	6.22	-	-	5.75	-	4.15	0.2	-
cis-isogenol	-	1.99	-	-	-	-	-	1.3	-	-	-
Linalool	-	1.79	-	-	-	-	-	2.3	-	-	-
Estragole	2.3	-	-	0.46	-	-	-	2.5	-	14.5	1.86
α-Terpineole	-	-	-	-	-	-	-	1.5	-	-	-
Fenchone	5	-	-	-	6.16	4.12	-	--	-	-	-
Longifolene	-	-	-	-	-	-	-	-	-	-	2.64
Zingiberene	-	-	0.11	-	-	-	0.59	-	-	-	1.06
Camphene	-	-	-	-	-	-	-	-	-	-	-
Limonene	-	-	-	-	-	-	-	-	-	-	-
Estallylanisole	-		-	-	-	-	-	-	-	-	-
Limonene	-	-	-	-	9.75	5.53	-	-	-	-	-
Estallylanisole	-	-	-	-	**76.70**	**85.28**	-	-	-	-	-

(-) : low proportion (<0.1%) or not detected.

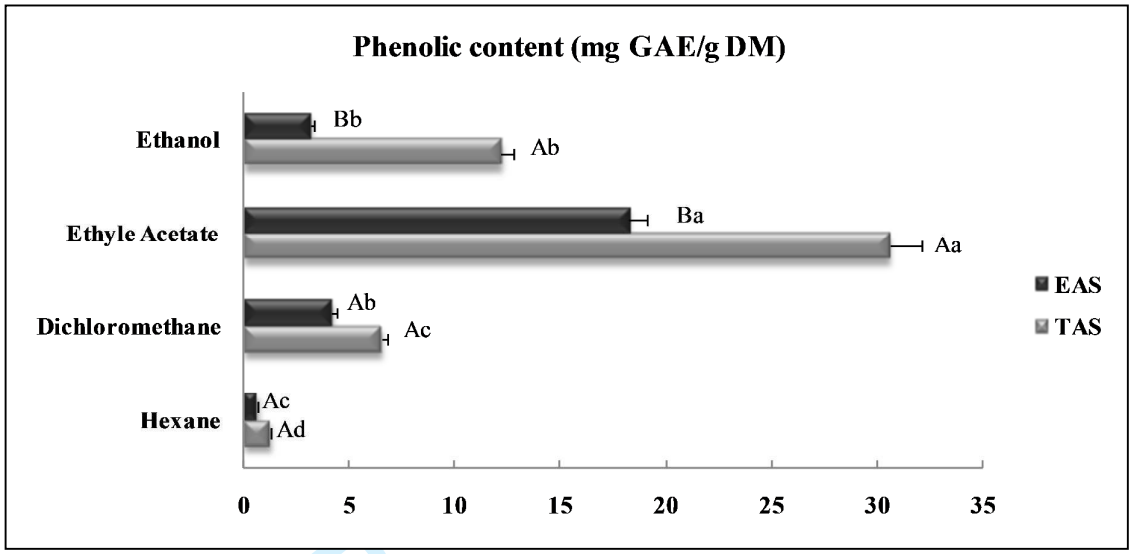


Figure S2. Total phenolic contents (mg GAE/g DM) of anise (*Pimpinella anisum*) seed extracts. The data marked with the different capital letter for provenance and small letter for the solvents in the table value share significant differences at $P< 0.05$ (Duncan test). Values are means of six replications (N=6±S.D); CE: catechin equivalents; TAS: Tunisian anise seeds; EAS: Egyptian anise seeds.

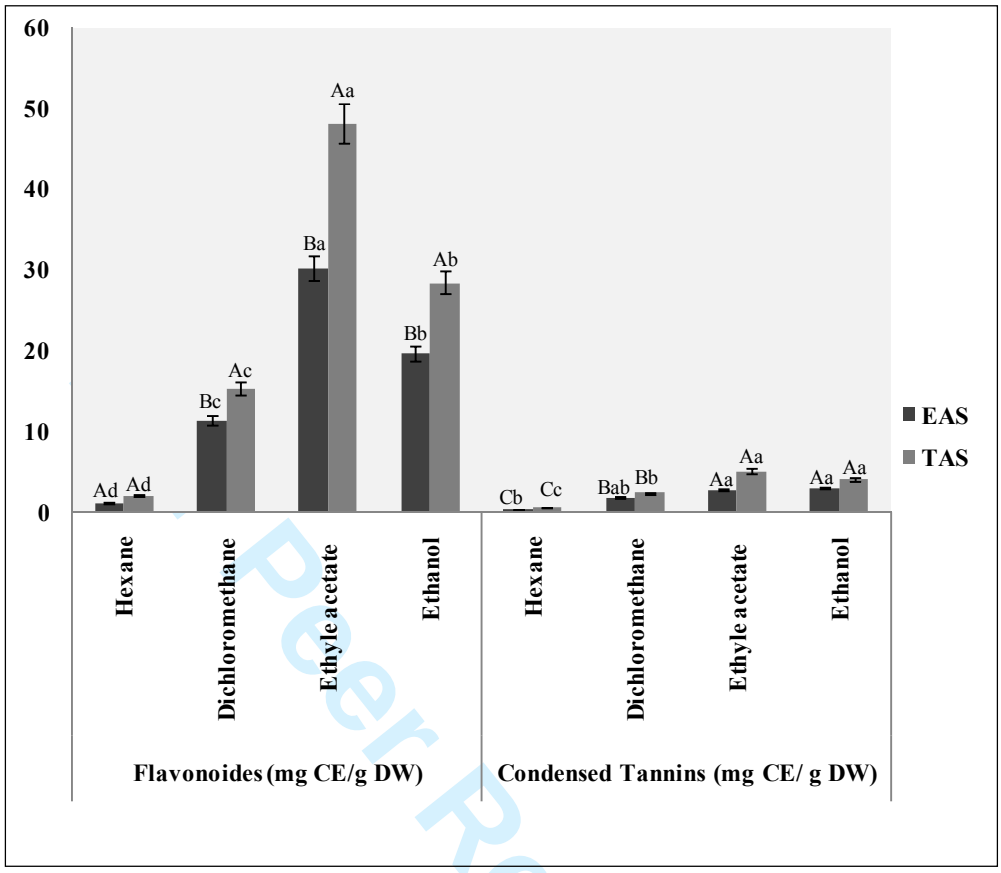


Figure S3. Total Flavonoid and tannin contents (mg CE/g DM) of anise (*Pimpinella anisum*) seed extracts. The data marked with the different capital letter for provenance and small letter for the solvents, in the table value share significant differences at $P< 0.05$ (Duncan test). Values are means of six replications (N=6±S.D); CE: catechin equivalents; TAS: Tunisian anise seeds; EAS: Egyptian anise seeds.

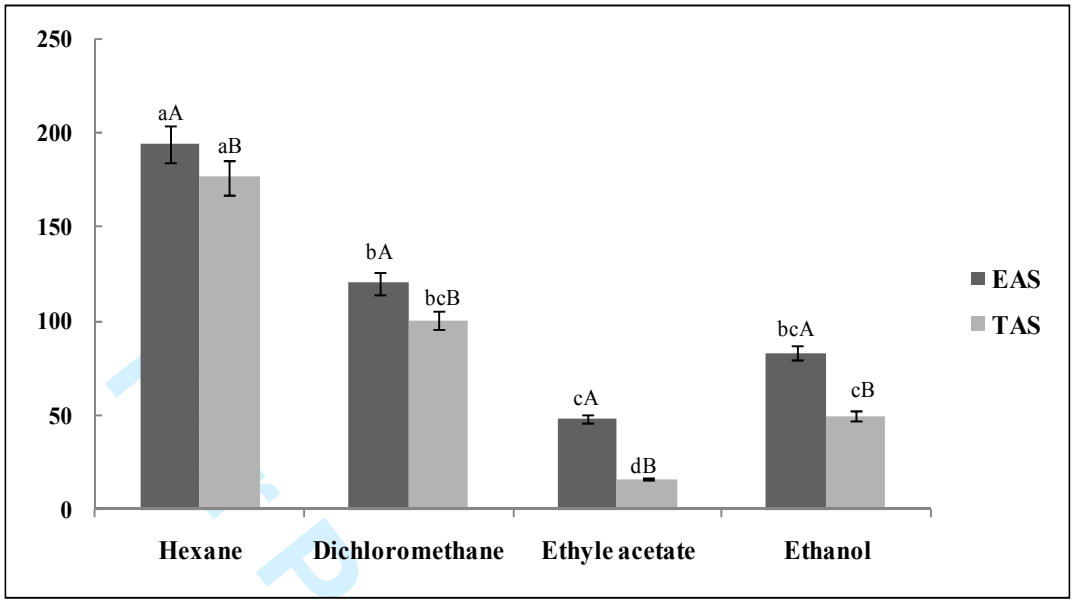
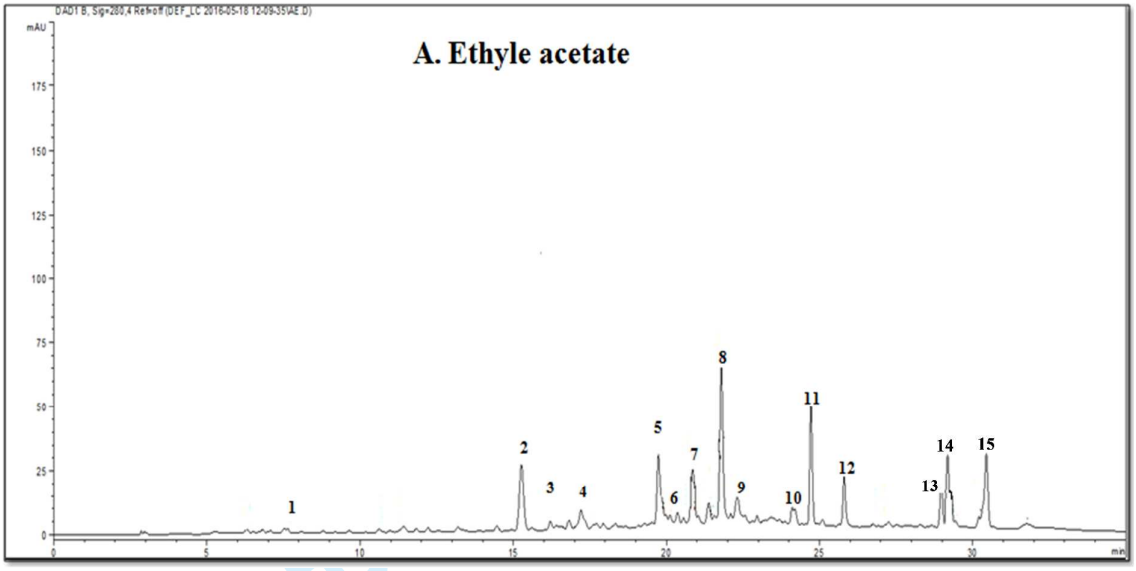
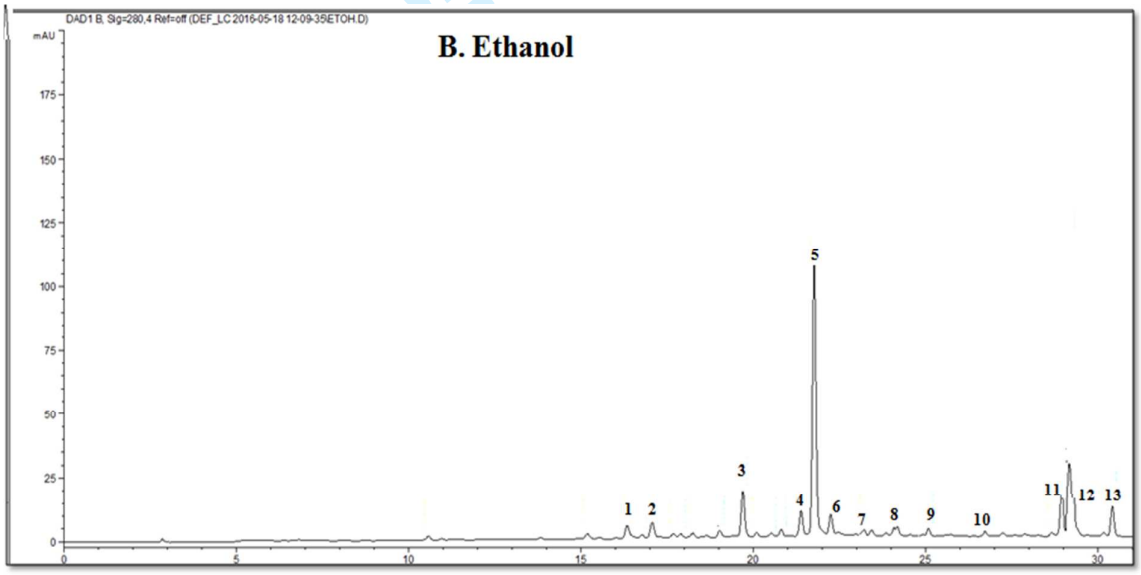


Figure S4. DPPH scavenging activity (IC50) of different seed extracts (TAS and EAS). Values are means of six replications (N=6±SD). The data marked with the different capital letter for the provenance and small letter for the solvent. In the histograms of each IC50 value share significant differences at $P<0.05$ (Duncan test).



1. Gallic acid; 2. Chlorogenic acid; 3. Epicatechin 3Θ gallate; 4.Syringic acid; 5. *p*-coumaric acid; 6. Coumarine; 7. Rutin ; 8. Rosmarinic acid; 9. Ellargic acid; 10. Quercetin; 11. Naringin; 12. Apigenin; 13. NI; 14: NI; 15: NI.



1. Epicatechin-3Θ gallate; 2. Caffeic acid; 3. *p*-coumaric acid; 4. Rutin; 5. Rosmarinic acid; 6. Ellargic acid; 7. Quercetin; 8. Naringin; 9. Larcitrin; 10. Cirsimartin; 11. NI; 12. NI; 13. NI.

Figure S5. Reverse-phase high -performance liquid chromatography (RP-HPLC) chromatographic profiles of the phenolic compound in ethyl acetate (A) and ethanol (B) extracts of anise (*Pimpinella anisum*) seeds monitored at 280 nm, NI; not identified.